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Uric acid substantially enhances the free radical-induced inactivation of alcohol dehydrogenase

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Lactate dehydrogenase (LDH) and yeast alcohol dehydrogenase (YADH) are inactivated when attacked by hydroxy free radicals ($OH^{\cdot}$). Organic molecules with a high rate constant of reaction with $OH^{\cdot}$ such as ascorbate or urate can compete with the enzymes for these strongly oxidising radicals. However, although 10^{-3} M ascorbate can substantially protect both LDH and YADH from $OH^{\cdot}$ attack, in the presence of 10^{-3} M urate only LDH is protected. In the case of YADH an even greater degree of inactivation than with $OH^{\cdot}$ occurs. The extent of inactivation is considerably reduced when oxygen is absent, in agreement with a urate peroxy radical perhaps being partly responsible for the increased inactivation of the enzyme.

Uric acid *Free radical* *Enzyme inactivation* *Oxygen toxicity*

1. INTRODUCTION

During the last decade much attention has been focussed on the mechanisms by which various agents can protect cells from the reactions of oxygen free radicals such as the hydroxyl radical, $OH^{\cdot}$, and the superoxide radical, $O_2^{\cdot -}$. The enzymes superoxide dismutase, catalase and glutathione peroxidase, the vitamins C and E, and beta carotene are thought to play important roles in this respect [1,2]. Authors in [3] recently suggested that uric acid may also play a role in protecting man from the damaging action of oxygen free radicals and may be partly responsible for man's relatively long lifespan. It is in this light that we have been reinvestigating the comparative protective capacity of ascorbate and uric acid. As anticipated, we have found that both compounds can protect the enzyme lactate dehydrogenase from free radical mediated damage. To our surprise, however, we have found that with alcohol dehydrogenase, the presence of uric acid can markedly enhance the damage induced.

2. MATERIALS AND METHODS

Oxygen radicals were generated by well documented radiation procedures [4–6]. Air-saturated solutions of the enzymes were irradiated using a Cobalt 60 gamma source (dose rate 1–10 $Gy \cdot min^{-1}$) in the absence and presence of uric acid (neutralised with Na_2HPO_4) and ascorbate. Solutions of lactate dehydrogenase (LDH, Boehringer Mannheim, hog muscle, 0.01 $mg \cdot ml^{-1}$), liver alcohol dehydrogenase (LADH, Sigma, horse, 0.1 $mg \cdot ml^{-1}$), and yeast alcohol dehydrogenase (YADH, Sigma, 0.1 $mg \cdot ml^{-1}$) were prepared in doubly distilled water immediately before use.

3. RESULTS AND DISCUSSION

The remaining activity measured when aerated control solutions of LDH and YADH were irradiated are shown in fig.1. Under these conditions any enzyme inactivation observed can be attributed principally to the reactions of the hydroxyl and superoxide free radicals. Since the superoxide radical has shown to be ineffective in inac-

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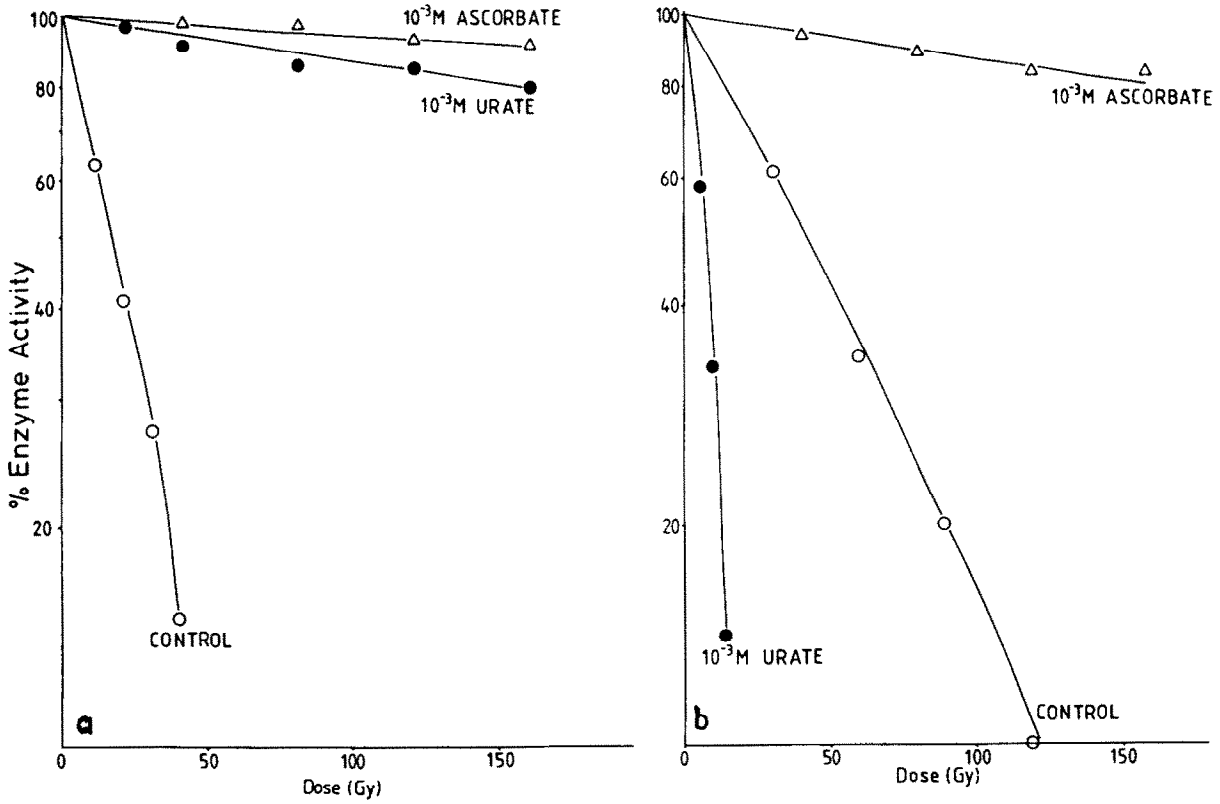

Fig.1. Radiation-induced inactivation of lactate dehydrogenase (a) and alcohol dehydrogenase (b) in the absence and presence of urate or ascorbate (1 mM).

tivating ADH, or LDH [7–9] the inactivation shown can be attributed principally to reactions of the hydroxyl radical.

Ascorbate and uric acid both react rapidly with $OH^{\cdot}$ [9,10]. It was therefore anticipated that the presence of either of these compounds in high concentrations at the time of irradiation would result in enzyme protection. With LDH, this was indeed the case (fig.1a). In the case of YADH and LADH, however, only ascorbate protected; in the presence of urate increased inactivation was observed (fig.1b). Neither urate nor ascorbate had any effect on the activity of the enzymes in the absence of radiation. This pointed to the radical from the reaction of $OH^{\cdot}$ with urate, probably following the further addition of oxygen, being responsible for the enzyme's inactivation. Indeed, in similar experiments with nitrogen-saturated solutions, the radiation dose, measured in grays (Gy) to reduce the activity to 37%, D_{37}, was considerably greater than when oxygen was present (table 1).

Table 1

Radiation-induced inactivation of yeast and liver alcohol dehydrogenase and lactate dehydrogenase in solution (pH 6.9 ± 0.1) in the absence and presence of urate or ascorbate (1 mM), expressed as D_{37} in Gray

System	YADH	LADH	LDH
Air	54	140	22
Urate/air	9	100	>500
Urate/nitrogen	205	>250	>1000
Ascorbate/air	>500	>1000	>1000

Clearly, ascorbate and urate do differ in their protective abilities depending on the particular biological system involved. Perhaps more importantly, this sensitising phenomenon may be not restricted to alcohol dehydrogenase. Studies with other enzymes and related purine and pyrimidine derivatives are in progress. Many 'repair enzymes' are known to be sensitive to oxidation and the fact

that their activity necessarily takes place in the vicinity of nucleic acid bases may be significant for injury generally.

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